THE EFFECT OF RESPONSE COST AND THE CONTROL OF SPECIES TYPICAL BEHAVIOR ON A DAILY TIME-PLACE LEARNING TASK

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The Effect of Response Cost and the Control of Species Typical Behavior on a Daily Time-Place Learning Task

by

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Abstract

Two theories, which have been hypothesized to mediate acquisition in daily time-place learning (TPL) tasks were investigated: the Response-Cost (RC) hypothesis and the Species-Typical Behaviours (STB) hypothesis. According to the RC hypothesis, rats only learn daily TPL tasks if there is high cost in either effort or time for making an incorrect choice. According to the STB hypothesis, rats learn the daily TPL tasks, however the intrusion of species typical behaviours such as patrolling mask evidence of this learning. Two experiments tested the validity of these hypotheses. Rats were trained that one lever at the end of one choice arm of a T-maze provided food in the morning and six hours later a lever in the other choice arm provided food. In Experiment 1, two groups tested the RC theory by manipulating the density of the reinforcement schedule used. A third group tested the importance of the STB by giving the rats time to patrol the maze prior to the start of the experiment. If only first arm choice data were considered there was little evidence of learning. However, both first press and percentage of presses on the correct lever, revealed evidence of TPL in all groups tested. Unexpectedly, the low response cost group performed better than the high response cost group and the species typical behaviour group performed the worst. To control for the fact that the high response cost group was on the maze for a longer period of time than the rats in the other two groups, a second experiment was conducted. Experiment 2 also used a low response cost group and a species typical behaviour group, except these animals remained on the maze for the same amount of time as the rats in the high response cost group from Experiment 1. The additional time on the maze in Experiment 2 did not have an effect on performance. Skip

session probe trials confirmed that the majority of the rats that acquired the task were using a circadian timing strategy. We outline two possible explanations which might account for the results from the present study.

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The Effect of Response Cost and the Control of Species Typical Behavior on a Daily Time-Place Learning Task

In nature, it is advantageous for an animal to be able to anticipate the spatiotemporal variability of a biologically significant event, such as food, a mate, or the threat of predation. For example, oystercatchers, a type of seabird, are known to travel long distances to specific beaches only during the times when the tide is low so that they can optimize mussel foraging (Daan & Koene, 1981). Gallistel (1990) posited that time-place-event memory codes are automatically formed for biologically significant events and that these codes can then be retrieved to guide an animal's behaviour during a current biological event. This theory has lead to the inception of time-place-learning (TPL) studies (See Thorpe & Wilkie, 2006 for a review), in which an animal must associate an event with a time and place to receive reinforcement.

There are two classes of TPL studies that are defined by the duration that is to be timed. In daily TPL, which is the focus of the present study, the location of the event varies depending on the time of day, whereas in interval TPL the location of the event varies depending on the duration of time since some external event, usually in the range of minutes to hours. For example, in daily TPL studies, food is located in one place in the morning and another place in the afternoon. In interval TPL studies, food is available at one of several places for a few minutes and then food availability switches to a different location.

To solve daily TPL tasks, animals can use a *circadian*, *interval, ordinal*, or *alternation* strategy (Carr & Wilkie 1997a). In *circadian timing,* the times of events that have a fixed periodicity are associated with different phase angles of an endogenous

circadian oscillator. The animal is then able to use this information to accurately predict the time that these events occur. An animal that is using an *interval timer* has learned that the event of interest occurs after a period of time since the occurrence of an external event, such as feeding or the turning on of the colony lights (see Pizzo & Crystal, 2002, 2006). An animal that is using an *ordinal timing* strategy has learned to anticipate the sequence of events that occur during a specific time period, but this does not necessarily mean that they have learned the exact time that these events have occurred. Finally, the animals can also acquire the tasks using a nontiming *alternation* strategy, which involves alternating the locations visited from trial to trial. Skipping one of the daily sessions and then analyzing the animals' behaviour in the next session can elucidate the type of strategy that the animals are using to complete the task.

Daily TPL has been documented in a variety of species including birds (garden warblers: Biebach, Gordijn, & Krebs, 1989; pigeons: Saksida & Wilkie, 1994), fish (inangas: Reebs, 1999; golden shiner: Reebs, 1996), honeybees (Wahl, 1932, as cited in Reebs, 1993), ants (Schatz, Beugnon, & Lachaud, 1994), mice (Van der Zee, Havekes, Barf, Hut, Nijholt, Jacobs, & Gerkema, 2008) and rats (See Thorpe & Wilkie, 2006 for a review). In the laboratory, daily TPL was first demonstrated with garden warblers (Biebach, Gordijn, & Krebs, 1989). In this study, food was available in one of four rooms located off of a central living chamber for 3 hr periods at four different times daily. The birds learned to enter the correct room at the appropriate time of day. The authors suggested that the birds were timing food availability because during probe trials in which no food was given the birds continued to visit the correct rooms at the appropriate times. It was concluded that the birds were using a circadian timer to solve the task

because changing the lighting conditions to constant illumination for 24 hours disrupted the birds' performance.

Krebs and Biebach (1989) further investigated the birds' timing strategy by preventing the birds from foraging in the first room that provided food daily. They found that 5 of the 9 birds were using an ordinal timer because when the first room was blocked during the first session, in the next session when given free access to all of the rooms, the animals chose to visit the first room instead of the room that should have been providing food (Room 2). Pigeons have also acquired time-place-event (TPE) associations in an operant box task that involved pecking at one key on one of four walls in the morning to receive food and pecking at another key on a different wall to receive food in the afternoon (Saksida & Wilkie, 1994). In this case, the birds were predominately using a circadian timer, although some of them appeared to be using an ordinal timer as well.

Carr and Wilkie (1997b) observed daily TPL in rats using a paradigm very similar to the pigeon operant task described above. In this operant box study, rats learned to press one lever in the morning and another lever in the afternoon to receive food. The authors determined that the majority of the rats were using an ordinal timer to solve the task. Mistlberger, de Groot, Bossert and Marchant (1996) also observed daily TPL using a slightly different paradigm, a T-maze with levers at the end of the choice arms. Skip session probes determined that the animals were using a circadian timing mechanism to determine which of the two levers provided food at the appropriate time. It should be noted that the dependent measure for this study was the percentage of correct presses to total presses because they did not find evidence of TPL if they just considered the animals' first arm choices. Several other studies have also demonstrated that rats are able

to solve daily TPL tasks in different paradigms, such as the open field maze (Pizzo & Crystal, 2002); T-maze with differential amounts of food available at each time (Thorpe & Wilkie, 2007); open field task with towers (Widman, Gordon, & Timberlake, 2000); and water maze tasks (Lukoyanov, Pereira, Mesquita, & Andrade, 2002; Widman, Sermina, & Genismore, 2004). However, in many of these studies the animals only acquired the task if specific conditions were met. For example, researchers in the Widman lab were only able to observe learning in the open field and water maze if the tasks were modified by adding towers to the open field and weights to the animals in the water maze (Widman et al., 2000; Widman et al., 2004). Similarly, Thorpe and Wilkie (2007) were only able to observe learning if different amounts of food were used for each session. These studies suggest that TPL only occurs under certain circumstances.

Furthermore, some studies have failed to find evidence of daily TPL in rats. For example, Thorpe, Bates, and Willkie (2003) investigated rats' ability to form TPE associations in a variety of tasks, such as the water maze, food rewarded place preference task, and radial arm maze. While the rats did learn the locations that provided food (place-event association), as evidenced by an increased tendency to go to those locations, they did not go to the correct locations at the correct time. However, in a go no-go task in which both of the arms provided food at one time and neither of the arms provided food at the other time, the rats did learn at which time of day food was present. Only a time-event association is required for successful completion of this task, rather than a time-place-event association. That is, the rats only had to learn at what times of day food would be available.

Other studies have also found it difficult to demonstrate TPL, even when using similar paradigms to those that have previously produced TPL. For example, using a paradigm similar to that used by Carr and Wilkie (1997a, b), Boulos and Logothetis (1990) found that only a few of their rats acquired daily TPE associations when two levers on opposite sides of a cylindrical chamber provided food at two different times daily. White and Timberlake also failed to find evidence of TPE associations in rats that were trained in a similar paradigm (1990, unpublished data cited from Widman et al., 2000).

Means, Ginn, Arolfo, and Pence (2000) did observe some TPL in a T-maze, but only 63 % of rats demonstrated TPE associations and only after many training trials. Furthermore, additional training post criterion (9 correct trials out of 10) for the animals that learned, actually caused performance to decline to 70%. In a follow up experiment, they manipulated various aspects of the procedure to try to ameliorate acquisition and performance (Means, Arolfo, Ginn, Pence, & Watson, 2000). They found that performance did not improve when one of the arms was made more distinct, when two trials were administered for each session, nor when one of the daily sessions was conducted in the light and the other was conducted in the dark. Furthermore, using natural light cycles or extinguishing repeated responding to one of the arms also did not improve performance (Means, Arolfo, Ginn, Pence, & Watson, 2000). However, similar to the findings of Thorpe et al. (2003), rats were able to acquire the go no-go task, suggesting that rats readily learn to discriminate the two different times of day.

Additionally, our lab (unpublished data, 2009) did not find any evidence of TPE associations on a plus maze, even when three trials were administered to the rats each

session. Similarly, White and Timberlake (1990) failed to observe daily TPL when food was provided in one of three arms in a radial arm maze during one of three specific times throughout the day.

There are two hypotheses that could account for the mixed results observed in daily TPL tasks involving rats. The first, the Response-Cost hypothesis, is based on the premise that rats only learn the task if the relative cost in energy or time that is associated with making an error (response cost) in a particular task is high (Widman et al., 2000). The second, the Species-Typical Behaviour hypothesis, instead argues that the rats do automatically learn the daily TPL task, regardless of response cost, but that the intrusion of species typical behaviours such as patrolling, hides the evidence of that learning. Thus, according to the first hypothesis, Gallistel's (1990) theory, which suggested that animals are able to automatically encode and retrieve time-place-event codes, is wrong. However, according to the second hypothesis, Gallistel's theory is correct and better measures of TPL need to be developed. I will first outline the evidence for each hypothesis and then detail a procedure for determining the veracity of each.

Bolous and Logothetis (1990) were the first to suggest that the poor performance demonstrated in their experiment might have been because the response cost of the task was minimal. For example, the distance between the two levers was very small and once the first reinforcer was obtained, a continuous reinforcement schedule was used. Similarly, response-cost also seems to account for rats' inability to learn the daily TPL tasks in the plus (unpublished data Thorpe, 2009) and radial arm mazes (Thorpe et al., 2003; White & Timberlake, 1990). In these tasks, the choice arms were located close

together and it did not take very much energy or time to travel to an alternative arm following an incorrect choice.

The Widman lab conducted several experiments focusing exclusively on the effect of an increased response cost on daily TPL tasks. First, they increased the response cost of an open field maze by adding vertical towers that contained food at the top of the towers (Widman et al., 2000). Using this modification 66% of the rats formed TPE associations and there was a positive correlation between the height of the towers and the likelihood of forming TPE associations. Response cost has also been investigated in water-maze versions of daily TPL tasks. Lukoyanov et al., (2002) first demonstrated that only severely food-deprived rats (received 60% of food eaten by ad libitum rats) could acquire TPE associations in the water-maze. Although the authors hypothesized that a food-entrained oscillator mediated successful performance, Widman et al. (2004) theorized that because the amount of food restriction was drastic, the cost of the task was higher for these animals because their caloric intake was depleted and the water maze is an energetically taxing task. With this in mind, Widman and colleagues (2004) increased the response cost in a daily TPL task in the water maze by adding weighted vests to the rats and they found that satiated rats could acquire TPE associations in this task.

Thorpe and Wilkie (2007) showed that rats could learn a low response cost daily TPL task if different amounts of reinforcers were given in each daily session. In their paradigm, rats were trained that in one daily session a large portion of food was available in one arm of a standard T-maze, while in the other session a small portion of food was available in the alternative arm. The rats learned to go to the correct location at the correct time of day, indicating that they were able to learn the TPE contingencies. These

authors hypothesized that in low response cost tasks rats learned two bipartite codes (Time-Event and Event-Place; see Figure 1). In typical paradigms the event is the same (e.g., one piece of Froot Loop™) and the rat learns that food is available at both times of day (i.e., T_1-E_{food} and T_2-E_{food}). The rat also learns that food is located in one of two places (i.e., E_{food}-P_1 and E_{food}-P_2). When the rat is placed on the maze at T_1 it knows that food is available, however food is equally likely to be in either P_1 or P_2 so it randomly distributes its choices between those locations. In the case in which different amounts of food are available in each session, the rat can easily use the differing "events" to solve the task (See Figure 1B). The authors speculated that in certain situations the rats were able to bind the two bipartite codes into a single tripartite code that would allow them to successfully solve the task. While, it seems that situations with high response cost allow the tripartite codes to be formed, the relation between response cost and TPL is not fully understood. For example, not all of the studies that have produced daily TPL have used a high response cost. Notably, Carr and Wilkie (1997a & b, 1999) used a VR-15 reinforcement schedule in their task, which could be argued to be of relatively low response cost and their rats did learn the task.

The second hypothesis that could account for poor performance in daily TPL tasks is that the intrusion of species typical behaviour may mask dependent measures such as the animal's first arm or lever choices (Thorpe, Jacova, & Wilkie, 2004). For example, when rats are placed in an operant box, they initially have a tendency to patrol the environment and press a variety of levers and this behaviour can mask any learning that has occurred. Carr and Wilkie (1997b, 199) implemented a 10-s nonreinforced period, in which lever presses were not counted at the start of every session. If these data

were included it appeared that the animals had not acquired the task. However, further analysis of the data after the time-out period indicated that the rats did acquire the task. Similarly, in the Mistlberger et al. (1996) study in which rats had to press on levers located at the ends of the arms in a t-maze, if only the animals' first arm choice data were used they would have concluded that the animals did not learn the discrimination. However, it was found that the animals focused most of their responding at the appropriate lever during the correct times, suggesting that the rats did acquire TPE associations.

The Species-Typical Behaviour theory suggests that animals acquire TPE associations in all circumstances, we just fail to detect them depending on the measures analyzed, whereas the Response-Cost theory states that the animals only learn in effortful circumstances. If the Response Cost is high, it is less likely that the animal will display the intrusive behaviours such as patrolling. However, it is possible that these theories are not mutually exclusive. To determine the effect that controlling for species typical behaviour and varying response cost have on a daily TPL task in rats, two experiments were conducted in which each of these was systematically varied. A paradigm similar to that used by Mistlberger et al. (1996), in which a lever located at an arm of a T-maze provided reinforcement in morning sessions and a lever located at the other arm provided reinforcement in afternoon sessions, was used. Response cost was manipulated by varying the ratio of reinforcement, while the effect of species typical behaviours was manipulated by allowing varying amounts of time to patrol the maze prior to the start of the session.

Experiment 1

To assess the relative importance of response cost rats were randomly assigned to one of two groups. One group of rats (VR2) was reinforced according to a variable ratio 2 (VR2) while the second group (VR30) was reinforced according to a higher response cost VR30 schedule. If response cost is an important factor in determining whether or not rats successfully learn the TPE discrimination, it was expected that the VR30 group would acquire the task more quickly than the VR2 group.

A third group (TO-VR2) was also reinforced on a VR2 schedule but had a 2-min time out at the start of each session. As in the Carr & Wilkie (1997b, 1999) studies the maze lights remained off and the responses of the rats had no effect on reinforcement. This allowed the rat time to patrol the maze and therefore control for the effect of species typical behaviours. Once the maze lights were turned on, the rat was reinforced for pressing according to a variable ratio two (VR2) schedule which is deemed to be of relatively low response cost. Therefore, this group has a low response-cost and a time-out period. If response cost is important, it was expected that the rats would not learn to go to the correct lever at the correct time of day. If however, the opportunity for the rat to patrol the maze is important, the inclusion of the 2-min time-out might allow us to detect evidence of task acquisition.

Method

Subjects and Apparatus

To make running the experiment more feasible we separated the 25 male Long Evans rats into two cohorts. All rats were obtained from Charles River (St. Constant, Quebec). The 12 rats in cohort one were approximately 57 days old at the start of training and approximately 104 days old at the start of discrimination training. One rat was

dropped from this cohort because it did not consistently press the levers. The 13 rats in cohort two were approximately 55 days old at the beginning of training and approximately 84 days old at the start of discrimination training. Two rats were dropped from the second cohort because one did not consistently press the levers and the other rat was ill.

All of the rats received a standard rat diet (PMI Nutrition International, MO, USA). Their weights were maintained at 85% of their free-feeding weight and the rats were allowed to gain approximately 5 g per week to allow for growth. The rats were fed every day at approximately 4:00 p.m., even on days that they were not tested. The rats were housed individually in transparent plastic cages (45 x 25 x 21 cm) that were lined with aspen woodchip bedding (Necto Company, New York, New York). The animals were also given paper cups twice weekly to make additional bedding. The rats were kept in a colony room that was maintained on a 12:12-h light dark cycle, with light onset at 7:00 a.m. and offset at 7:00 p.m. During pretraining and discrimination training, 45 mg pellets (Bio Serv, Frenchtown, NJ) were used as reinforcers. The rats had free access to water at all times, except during experimental sessions.

Before and during training the rats were handled extensively and all of the rats received 20-min sessions in an enriched environment, approximately three times a week. All of the rats were enriched individually, except for the rats in cohort two which were enriched in pairs for the first two weeks in the colony. The enriched environment consisted of a Plexiglas enrichment box (61 cm x 61 cm x 61 cm) that was lined with aspen woodchip bedding (Necto Company, New York, New York) and contained several plastic tubes and containers as well as a standard running wheel.

The animals were trained to lever press in a Plexiglas operant conditioning box (47 cm x 47 cm x 32 cm) that had a retractable lever (Med Associates Inc, St. Albans, Vermont), in the center of each of the four walls of the box. Pellet dispensers (Model ENV-203045, Med Associates, Inc., St. Alban, Vermont, USA) were used to deliver the 45 mg pellets (Bio Serv, Frenchtown, NJ) to food wells that were mounted 6 cm from the floor. The box was lined with aspen woodchip bedding (Necto Company, New York, New York). The operant conditioning box was located in a room (170 cm x 160 cm) that contained a cabinet, a radio and a door.

A painted wood T-maze with non-retractable levers (Model ENV-110M, Med Associates, Inc., St. Alban, Vermont, USA) attached at each end of the choice arms was used during discrimination training. Each arm of the T-Maze was 53.5 cm x 15.0 cm and the T-maze was elevated 84.0 cm above the floor. Plexiglas walls were attached to the end of each of the choice arms so that they could each support a lever, food cup, light, and pellet dispenser. These components were arranged in the same way as in the operant box that was used for shaping. The food cup was located 6.0 cm above the T-maze, while the lever and light were located 8.0 and 15.0 cm above the T-maze respectively. The pellet dispenser was located 28.0 cm above the T-maze. An in-house designed controller box and computer program (python) were used to run the maze and collect the data. The T-maze was located in a room (604.0 cm x 248.0 cm) that contained two tables, a window, a sink with a cabinet, two doors, a poster, and a radio.

Procedure

Pretraining.

Rats were randomly assigned to one of three groups: VR30 (n = 7), VR2 (n = 8), and TO-VR2 (n = 7). For the VR30 group, there were three rats from cohort one and four rats from cohort two. For the VR2 group, there were four rats from each of the cohorts. Finally, for the TO-VR2 group there were four rats from cohort one and three rats from cohort two. The rats were first taught to lever press in the operant conditioning box. Only one lever was available at a time and its wall location varied across days. Rats in all three groups were initially shaped to a VR30 schedule of reinforcement. This training took an average of 17 days. It should be noted that for the first three sessions in the operant box the rats in cohort two were pretrained in pairs and in these instances two levers were available.

Once rats were successfully pressing on a VR30 schedule in the operant box, they began habituation sessions on the T-maze. The rats received several habituation sessions until they were pressing the levers and eating rewards on the maze. Once the rats were habituated to the maze, the rats' received additional training on the maze to ensure that they responded on both levers under a CRF schedule of reinforcement. All of these pre-training sessions were conducted at times different than the eventual discrimination training times.

Finally, the rats received one week of pretraining in which they received two daily sessions as in the discrimination training (see next paragraph). In this phase, the incorrect lever was blocked and they were reinforced according to the appropriate reinforcement schedule for their assigned group.

Discrimination training.

Discrimination training then began and the animals were tested twice daily, 5 days a week, for a total of 70 days (fourteen 5-day blocks). The testing began at 8:30 a.m. and 2:30 p.m. One lever provided reinforcement in morning sessions and the other lever in afternoon sessions. The morning and afternoon locations were counterbalanced across rats. Rats were tested individually and in the same order each session. Rats were held in their home cages on a cart in the experimental room while they awaited their turn to be tested.

To begin each session the rat was placed on the end of the stem of the T-maze and the corresponding computer program for that rat was started immediately. Rats in the VR30 group were on the maze for 10 min each session. Rats in the VR2 and TO-VR2 groups were yoked to a partner in the VR30 groups such that they received the same number of pellets. For the VR30 and VR2 groups, the lights were turned on immediately when the trial was started. However, for the TO-VR2 rats, the lights were not turned on until the 2-min time-out period had elapsed and although the levers were accessible during the time-out period, presses did not count until this period had elapsed. Also, for all groups reinforcement was contingent on presses on the correct lever, but all presses were recorded with 0.2-s accuracy by the computer.

Various dependent measures were used including the rat's first arm choice (entire body minus the tail in an arm), first press, and the percentage of presses on the correct lever compared to the incorrect lever before the first reinforcer was administered (referred to as pre-reinforcement presses data). The computer automatically recorded all of the lever presses, whereas the rat's first arm choice was recorded manually by an experimenter that observed the rats' behaviour through a doorway into the experiment

room. For the first cohort, the rats' first arm choice data were not recorded until 19 days into discrimination training due to a procedural error. Thus, for the first arm choice data we only considered the last 10 blocks (5 days per block) of the experiment for both cohorts.

To examine the effect of training over time, the data were grouped into blocks of 5 days (five morning sessions and five afternoon sessions). Each of the dependent measures was calculated as a percentage of trials that was correct on that measure within each of the blocks.

An animal was considered to have learned the task when it had achieved a criterion of 18/20 correct trials. This criterion was calculated for each of the three measures. The animal's first press and pre-reinforcement presses data were analyzed separately. When considering the pre-reinforcement presses data a trial was coded as correct if the percentage of presses on the correct lever compared to the incorrect lever was greater than 50 %.

Skipped session probes.

To determine whether rats were relying on a circadian, ordinal or alternation strategy to solve the task, probe sessions were conducted in which morning or afternoon sessions were omitted and performance on the subsequent session was analyzed. If the animals were using a *circadian* strategy then they should always chose the correct location in the session following the omitted one, regardless of whether an AM or a PM session was skipped. If the animals were using an *ordinal* strategy then they should have gone to the morning location when a PM session was skipped, but when an AM session was skipped they should have incorrectly gone to the morning location in the PM session.

If the animals were using an *alternation strategy* then they should always go to the incorrect location regardless of which session was skipped.

These probe sessions were conducted once an animal had achieved criterion in any of the three measures. To ensure that there was enough data to analyze the pre-reinforcement presses measure, on the probe trials following the skipped session the rat had to respond on the correct lever a minimum of five times before a reinforcer was given. A total of six skip session probe trials were conducted (three morning and three afternoon trials) for each animal. Only one probe was conducted a week and it was only administered if the animal had been run the day before.

Results and Discussion

Data were only included in the analyses if the animals had been tested in both sessions (morning and afternoon) that day[1]. Furthermore, the data for probe trial days were not included in the analyses of the discrimination training data for all three of the measures. This resulted in either one or two days of data being omitted per probe trial depending on if the morning or afternoon session was skipped. Since the Block factor follows a continuum indicating the passage of time, this factor was analyzed using trend analyses, and only the linear and quadratic effects of the Block factor and interactions involving this factor are reported. Also for the TO-VR2 group, the animals' first arm choice was recorded when the animals were first placed on the maze (i.e., during the time-out), but the first press and percentage of presses on the correct lever data were only

[1] It was very rare for a rat not to have been run in both the morning and afternoon. It happened a maximum of 3 times for any one rat.

analyzed after the two minute time-out period had elapsed. Finally, because there were so many one sample t-tests conducted (30 for the first arm choice data and 42 each for the first press and pre-reinforcement presses data), the alpha was reduced to .005 to control for inflated Family-Wise Error for all of the t-tests.

First Arm Choice

Because of a procedural error, the first arm choice was only recorded after the 19th session for the first cohort of rats. Thus, the first arm choice was only analyzed for the final 10 blocks of the training (i.e., Blocks 5 to 14). Also, because there were some missing values for the first arm choice data, each block was calculated by taking an average of the data for the available days, and the missing days were not included. As a result some of the blocks did not contain all 10 data points (i.e., five from the morning and five from the afternoon). For the VR30, VR2, and TO-VR2 groups there was an average of 96, 96, and 97 of the total 100 data points, respectively for the entire experiment. It should also be noted that, we included the first arm choice data for the TO-VR2 rats for completeness sake, however given our hypothesis that the time-out allowed the rats to patrol the maze, it was expected that the first arm choice would be at chance levels for these rats.

When considering the first arm choice data, only one of the rats (VR2 group) reached a criterion of 18/20 correct trials at any point during the final 10 blocks of the experiment. This rat reached criterion at day 50 of discrimination training.

A 10 (Block; Blocks 5 to 14) X 2 (Time of Day; morning vs afternoon) X 3 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average

percentage of choices to the correct arm per block. The analyses indicated that there was a linear (F (1, 19) = 4.43, p = .049) effect for Block, but there was not a quadratic effect (F (1, 19) = 0.05, p = .824) (Refer to Figure 2A). Nor was there a linear Group X Block interaction (F (2, 19) = 0.43, p = .656). However, there was a main effect of Group (F (2, 19) = 4.85, p = .020) and Time of Day (F (1, 19) = 5.18, p = .036). The rats performed better in the afternoon (M = 64.70) than in the morning (M = 43.66). Regarding the differences in the Group factor, Tukey's post hoc tests indicated that the VR2 group (M = 60.05) performed better than the TO-VR2 group (M = 47.79) and this was the only significant difference among the groups.

One-sample t-tests were also conducted for each group to determine in which blocks the percentage of correct responses differed from chance (50 %). For the VR30 group, performance was not statistically greater than chance for any of the blocks (Block 14: M = 63.21; t (6) = 2.06, p = .085). For the VR2 rats, performance was also not statistically greater than chance for any of the blocks, however three of the last four blocks were approaching significance (Block 14: M = 67.81; t (6) = 2.74, p = .029). Similarly, for the TO-VR2 group performance was not statistically greater than chance for any of the blocks (Block 14: M = 50.36; t (6) = 0.08, p = .936).

Generally, the first arm choice data suggest that the animals did not acquire the discrimination. For example, only one of the animals achieved criterion. Even though the ANOVA indicated that performance did improve across blocks, all of the t-tests for all the groups indicated that performance was not statistically greater than chance in any of the blocks. However, performance approached significance by Block 14 for the VR2

group. Contrary to our expectations, the VR2 group chose the correct arm more than the TO-VR2 group.

First Press

When considering the first press data all seven of the rats in the VR30 group (M = 41 days), seven of the eight rats in the VR2 group (M = 36 days), and five of seven rats in the TO-VR2 (M = 54 days) group reached criterion during the 14 blocks of the experiment.

A 14 (Block; Blocks 1 to 14) X 2 (Time of Day; morning vs afternoon) X 3 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average percentage of correct first lever presses per block. The analysis indicated that there was a linear (F (1, 19) = 82.31, p < .001) and quadratic effect (F (1, 19) = 17.85, p < .001) (Refer to Figure 3A). There was also a main effect for Time of Day (F (1, 19) = 7.50, p = .013), with the animals performing better in the afternoon (M = 79.46) than in the morning (M = 71.45). However, there was not a main effect for Group (VR30: M = 78.47; VR2: M = 79.02; TO-VR2: M = 68.88) (F (2, 19) = 3.31, p = .059), nor was there a linear Group X Block interaction (F (2, 19) = 2.71, p = .092).

One-sample t-tests were also conducted for each group to determine in which blocks the percentage of correct responses differed from chance (50 %). For the VR30 group performance was statistically greater than chance in all of the blocks after Block 5 (smallest significant t value Block 8: M = 85.71; t (6) = 5.21, p = .002). For the VR2 group performance was statistically greater than chance in all of the blocks except for the first two (smallest significant t value Block 3: M = 78.75; t (7) = 4.50, p < .001). For the

TO-VR2 group performance was only statistically greater than chance in three of the blocks (Blocks 5, 11, and 12) (smallest significant t value Block 5: $M = 77.14$; $t\,(6) = 5.20$, $p = .002$).

Overall, the first press data provide evidence that the rats had learned the task. Even though the group effect only approached significance there is evidence to suggest that the VR30 and VR2 groups performed better than the TO-VR2 group. This claim is strengthened by the fact that the groups without a time-out were consistently better than chance starting from early in the training, while the TO-VR2 group did not appear to have acquired the task by the end of the experiment.

Pre-Reinforcement Presses

All of the rats in the VR30 ($M = 25$ days) and VR2 ($M = 29$ days) groups achieved criterion, but only five of the seven rats in the TO-VR2 ($M = 51$ days) group achieved criterion when considering the pre-reinforcement presses. Both of the rats that failed to reach criterion when using this measure, also failed to reach criterion when considering the first press data.

A 14 (Block; Blocks 1 to 14) X 2 (Time of Day; morning vs afternoon) X 3 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average percentage of presses on the correct lever before reinforcement, per block. The analysis of the Block factor indicated that there was a linear ($F\,(1, 19) = 84.14$, $p < .001$) and quadratic effect ($F\,(1, 19) = 55.64$, $p < .001$) (Refer to Figure 4A). There was a main effect for Time ($F\,(1, 19) = 5.16$, $p = .035$), with the animals performing better in the afternoon ($M = 84.58$) than the morning ($M = 79.26$). There was also a main effect of

Group (F (2, 19) = 3.59, p = .048) and a linear Block X Group interaction (F (2, 19) = 3.64, p = .046).

Because there was a linear Block X Group interaction, follow up simple main effects (repeated measures) analyses were conducted for each group. For the VR30 group there was a linear effect for the Block factor (F (1, 6) = 29.57, p = .002, ψ = 1351.74, slope = 2.97). For the VR2 group there was also a linear effect for the Block factor (F (1, 7) = 23.71, p = .002, ψ = 718.58, slope = 1.58). For the TO-VR2 group there was also a linear effect for the Block factor (F (1, 6) = 38.11, p = .001, ψ = 792.75, slope=1.74). Although the linear effects for each group were statistically significant, the significant linear Block x Group interaction, illustrated by the differing slopes, suggests that the VR30 group learned the task quicker than the TO-VR2 and VR2 groups. However, this conclusion must be tempered by the fact that the VR2 group had a higher percentage correct in the first block than the other two groups.

One-sample t-tests were also conducted for each group to determine in which blocks the percentages differed from chance (50 %). For the VR30 group performance was statistically greater than chance in all of the blocks except for the first four (smallest significant t value Block 5: M = 86.51; t (6) = 6.44, p = .001). For the VR2 group performance was statistically greater than chance in all of the blocks except for the first one (smallest significant t value Block 2: M = 73.83; t (7) = 7.041, $p < .001$). For the TO-VR2 group performance was statistically greater than chance in all of the blocks except for the first three (smallest significant t value Block 14: M = 77.99, t (6) = 4.36, p = .005).

As with the first press data, the pre-reinforcement presses data suggest that the animals acquired the task. All but two of the rats achieved criterion. The significant linear

Block x Group interaction suggests that there is a difference in the groups in terms of how quickly they acquired the task. The linear slope values suggest that the VR30 group might have acquired the task the quickest, but this is in contrast to the one-sample t-test data showing that the rats in the VR2 group learned the task the quickest. The different interpretations are likely due to the quadratic nature of the data and the fact that the VR2 group had a higher starting point than the other two groups.

Skipped Session Probes

To determine which strategy the rats were using to solve the task, accuracy on the sessions following a skipped session were analyzed. If the rats tended to be correct following both skipped morning and skipped afternoon sessions, they were labeled Circadian timers. If the rats tended to be correct following skipped afternoon sessions, but incorrect following skipped morning sessions they were labeled Ordinal timers. And if they were incorrect following both types of skipped sessions they were labeled Alternators. In the VR30 group, 4 rats used a circadian strategy and 3 rats used an ordinal strategy. In the VR2 group, 7 rats used a circadian strategy and 1 rat used an alternation strategy. And in the TO-VR2 group, 4 rats used a circadian strategy and 1 rat used an ordinal strategy. Overall, the majority of rats (15/20 rats that received probe trials) used a circadian strategy.

Experiment 2

Based on both the first press and pre-reinforcement presses data, rats in the VR2 group performed better than rats in either the VR30 or the TO-VR2 group, when one considered the block in which they first performed significantly better than chance. Furthermore, when considering the first press data, the rats in the VR2 group achieved

criterion in fewer days than the VR30 group. This is surprising because rats in the VR2 group had a lower response cost and did not have an opportunity to patrol the maze prior to the start of the session. However, there was a major procedural difference between the VR30 group and the other two groups. While the number of pellets received by the rats in the three groups was equal, rats in the VR30 group spent significantly more time on the maze than rats in the other two groups. For example some of the VR2 rats in Experiment 1 were on the maze for less than 2-min. It is possible that the VR2 rats performed better than the rats in VR30 group because they were on the maze for a shorter period of time. Thus, to provide further evidence that the higher response cost does not improve performance in the daily TPL task, we needed to make sure that the rats in the VR2 group would still perform in a similar fashion if they were exposed to the maze for 10-min. Therefore, in the second experiment, VR2 and TO-VR2 groups were again used, but instead of being yoked, the rats in these groups were on the maze for 10-min. In the TO-VR2 10-min group the rats had 10-min on the maze in addition to the 2-min time-out period at the start of every session. The two groups from Experiment 2 were added to those of Experiment 1 to determine if the addition of these groups had an effect on the overall results and so that comparisons could be made between the different groups that were used in both the experiments.

Method

Subjects and Apparatus

Rats were randomly assigned to either the VR2 10-min (n = 4) or TO-VR2 10-min (n = 4) group. We only used four rats per group in this experiment because this was just a preliminary experiment and given the fact that there was almost a ceiling effect for

the VR2 and TO-VR2 rats from Experiment 1 it would have been very difficult for the rats in Experiment 2 to do better. As in Experiment 1 half of the rats in each group were run in the first cohort and the other half in the second cohort. All rats were male Long Evans and were obtained from Charles River (St. Constant, Quebec). The rats in cohort one were approximately 57 days old at the start of training and approximately 104 days old at the start of discrimination training. The rats in cohort two were approximately 55 days old at the beginning of training and approximately 84 days old at the start of discrimination training. The rats were housed and cared for in the same manner as in Experiment 1. The same apparatuses that were used in Experiment 1 were used in Experiment 2.

Procedure

Pretraining and habituation procedures were identical to those in Experiment 1. The VR2 10-min and TO-VR2 10-min groups were the same as their counterparts from Experiment 1, except that the rats remained on the maze for 10-min instead of being yoked to the rats in the VR30 group. Although these new groups were equivalent in terms of time on the maze, the rats in these groups received a lot more pellets than those in the VR30 group from Experiment 1 (max of approximately 150 pellets for the VR2 10-min and TO-VR2 10-min groups compared to approximately 20 pellets for the VR30 group). As in Experiment 1 the TO-VR2 10-min group also had a 2-min time-out period at the start of every session, but in this case the time-out period was followed by 10-minutes in which the levers were active. Discrimination training was exactly the same as in Experiment 1 and the rats were run for 70 days.

The same dependent measures (first arm choice, first press, and pre-reinforcement presses) that were used in Experiment 1 were used in Experiment 2. As in Experiment 1, for the first cohort we did not start to collect the animals first arm choice data until 19 days into discrimination training. Thus, for the first arm choice data we only considered the last ten blocks (5 days per block) of the experiment for both cohorts. As in Experiment 1, an animal was considered to have acquired the task if they achieved a criterion of 18/20 correct trials in any of the three measures and skip probe trials were administered to the rats that had acquired the task.

Results and Discussion

Data were only included in the analyses if the animals had completed both sessions (morning and afternoon) that day[2]. Furthermore, the data for probe trial days were not included in the analyses of the discrimination training data for all three of the measures. This resulted in either one or two days of data being omitted per probe trial depending on if the morning or afternoon session was skipped. Data from this Experiment were added to the data in Experiment 1 and omnibus analyses were conducted. All other aspects of the data analysis were the same as in Experiment 1.

First Arm Choice

Because of the same procedural error mentioned in Experiment 1, the first arm choice was only recorded after the 19th session for the first cohort of rats. Therefore, first arm choice was only analyzed for the final 10 blocks of the training. Also, because there were some missing values for the first arm choice data each block was calculated by

[2] It was a very rare for a rat not to have been run in both the morning and afternoon. It happened a maximum of 3 times for any one rat.

taking an average of the data for the available days and the missing days were not included. As a result some of the blocks did not contain all 10 data points (five from the morning and five from the afternoon). For both the VR-2-10-min and TO-VR2-10-min groups the average data points obtained were 92 of the total 100 for the entire experiment. It should also be noted that, we included the first arm choice data for the TO-VR2 10-min rats for completeness sake, however given our hypothesis that the time-out allowed the rats to patrol the maze, it was expected that the first arm choice would be at chance levels for these rats.

When considering the first arm choice data, none of the rats in either the VR2 10-min or the TO-VR2 10-min groups reached a criterion of 18/20 correct trials at any point during the final 10 blocks of the experiment.

A 10 (Block; Blocks 5 to 14) X 2 (Time of Day; morning vs afternoon) X 5 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average percentage of correct first arm choices per block. The analyses indicated that there was not a linear (F (1, 25) = 1.47, p = .237) or quadratic (F (1, 25) = 0.13, p = .725) effect for Block (Refer to Figure 2A & 2B). Nor was there a linear Group X Block interaction (F (4, 25) = 0.92, p = .467). However, there was a main effect of Time of Day (F (1, 225) = 18.42, $p < .001$), with the animals performing better in the afternoon (M = 69.98) than the morning (M = 37.72). There was also a main effect of Group (F (4, 25) = 3.78, p = .016).

To determine how the two new control groups would compare to the groups from Experiment 1, contrasts were designed to test for the following overall group differences: 1) VR2 vs. VR2 10-min; 2) TO-VR2 vs. TO-VR2 10-min; and, 3) VR30 vs. VR2 10-min

and TO-VR2 10-min combined. These contrast tests indicated that there was not a difference in performance between the VR2 ($M = 60.05$) and VR2 10-min ($M = 58.48$) groups ($F(1, 25) = 0.13$, $p = .723$), nor was there between TO-VR2 ($M = 47.79$) and TO-VR2 10-min ($M = 48.25$) groups ($F(1, 25) = 0.01$, $p = .919$). Also, there was not a difference in performance when the VR30 ($M = 54.70$) group was compared to both the VR2 10-min and TO-VR2 10-min groups combined ($F(1, 25) = 0.13$, $p = .722$).

One-sample t-tests were also conducted on the first arm choice data for each group to determine in which blocks the percentage of correct responses differed from chance (50 %). For the VR2 10-min rats, performance was not statistically greater than chance in any of the blocks (block 14: $M = 58.75$, $t(3) = 1.48$, $p = .235$). Similarly, for the TO-VR2 10-min group performance was not statistically greater than chance for any of the blocks (block 14: $M = 45.00$; $t(3) = -0.58$, $p = .604$).

As in Experiment 1, the first arm choice data in the second experiment suggest that the animals' did not acquire the task. None of the rats in the VR2-10-min or TO-VR2-10-min groups achieved criterion. The addition of the two new groups to those from Experiment 1, slightly changed the ANOVA results from Experiment 1, because there was not a linear effect of Block when all five groups were included. Although there was a Group difference, there were no differences in performance between the new groups and their counterparts from Experiment 1. The main effect of Time of Day suggests that the animals perform better in afternoon sessions than morning sessions.

First Press

When considering the first press data, all of the rats in the VR2 10-min ($M = 27$ days) group and two of the four rats in the TO-VR2 10-min ($M = 50$ days) group reached criterion during the 14 blocks of the experiment.

A 14 (Block; Blocks 1 to 14) X 2 (Time of Day; morning vs afternoon) X 5 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average percentage of presses on the correct lever per block. The analysis indicated that there was a linear ($F(1, 25) = 103.80, p < .001$) and quadratic effect ($F(1, 25) = 34.05, p < .001$) (Refer to Figure 3A and 3B). But, there was not a main effect for Time of Day ($F(1, 325) = 0.91, p = .350$), nor was there a main effect for group (VR30: $M = 78.47$; VR2: $M = 79.02$; TO-VR2: $M = 68.88$; VR2 10-min: $M = 82.86$; TO-VR2 10-min: $M = 70.36$) ($F(4, 25) = 2.72, p = .053$). However, the linear Block X Group interaction was significant ($F(4, 25) = 3.22, p = .029$).

Because there was a Block X Group interaction, follow up simple main effects (repeated measures) analyses were conducted for each group. For the VR30 group there was a linear effect for the Block factor ($F(1, 6) = 51.71, p < .001, \psi = 1481.43$, slope = 3.26). For the VR2 group there was also a linear effect for the Block factor ($F(1, 7) = 14.62, p = .007, \psi = 856.25$, slope = 1.88). For the TO-VR2 group there was also a linear effect for the Block factor ($F(1, 6) = 25.87, p = .002, \psi = 921.42$, slope = 2.03). For the VR2 10-min group there was also a linear effect for the Block factor ($F(1, 3) = 54.77, p = .005, \psi = 1305.00$, slope = 2.87). For the TO-VR2 10-min group, however, there was not a significant linear effect for the Block factor ($F(1, 3) = 8.11, p = .065$). This suggests that the TO-VR2 10-min group might not have acquired the task. Also, the

linear slope values suggested that VR30 group might have acquired the task the fastest, followed by the VR2 10-min group.

One-sample t-tests were also conducted on the first press data for the VR2 10-min and the TO-VR2 10-min groups to determine in which blocks the percentage of correct responses differed from chance (50 %). For the VR2 10-min group, performance was statistically greater than chance in 8 blocks. After Block 3, only Blocks 6, 7, and 12 were not significant (smallest significant t value Block 9: $M = 87.50$; $t\ (3) = 7.83$, $p = .004$). Finally, for the TO-VR2 10-min group, performance was not statistically greater than chance in any of the blocks (Block 14: $M = 62.50$; $t\ (3) = 1.99$, $p = .141$).

These results suggest that the VR2 10-min animals acquired the task while the TO-VR2 10-min animals did not. The addition of the VR2 10-min and TO-VR2 10-min groups to those from Experiment 1 did change some of the ANOVA results that were found in the first experiment. Although there were still linear and quadratic effects, in this analysis there was also a linear Group X Block interaction and there was no Time of Day effect. Even though the Group effect only approached significance, the Group X Block interaction suggested that there were some differences in the rate of task acquisition. The TO-VR2 10-min group did not have a significant linear effect when tested by itself and none of the blocks were statistically greater than chance. This suggests that the TO-VR2 rats did not acquire the task. However, the VR2 10-min group had a significant linear effect and most of the blocks were statistically greater than chance, suggesting that these animals' acquired the task.

Pre-Reinforcement Presses

When considering the percentage of presses to the correct lever prior to the first reinforcement all of the rats reached the criterion (VR2 10-min: $M = 30$ days; TO-VR2 10-min: $M = 35$ days).

A 14 (Block; Blocks 1 to 14) X 2 (Time of Day; morning vs afternoon) X 5 (Group) mixed-model ANOVA was conducted with Block and Time of Day as Within factors and Group as the Between factor. The dependent measure was the average percentage of presses on the correct lever prior to the first reinforcer, per block. The analyses indicated that there was a linear ($F(1, 25) = 104.46, p < .001$) and quadratic effect ($F(1, 25) = 77.25, p < .001$) (Refer to Figure 4A and 4B). There was not a main effect for Time of Day ($F(1, 325) = 0.47, p = .498$), however there was a main effect of Group ($F(4, 25) = 3.04, p = .036$). There was also a significant linear Block X Group interaction ($F(4, 25) = 3.09, p = .034$).

Because there was a Block X Group interaction, follow up simple main effects (repeated measures) analyses were conducted for each group. For the VR30 group there was a linear effect for the Block factor ($F(1, 6) = 29.57, p = .002, \psi = 1351.74$, slope = 2.97). For the VR2 group there was also a linear effect for the Block factor ($F(1, 7) = 23.72, p = .002, \psi = 718.58$, slope = 1.58). For the TO-VR2 group there was also a linear effect for the Block factor ($F(1, 6) = 38.11, p = .001, \psi = 792.75$, slope = 1.74). For the VR2 10-min group there was also a linear effect for the Block factor ($F(1, 3) = 102.17, p = .002, \psi = 1035.48$, slope = 2.28). For the TO-VR2 10-min group there was not a significant linear effect for the Block factor ($F(1, 3) = 6.71, p = .081$). This suggests that all of the groups acquired the task except for the TO-VR2 10-min group.

One-sample t-tests were also conducted on the pre-reinforcement presses data for the VR2 10-min and the TO-VR2 10-min groups to determine in which blocks the percentage of correct responses differed from chance (50 %). For the VR2 10-min group, except for Block 6, performance was statistically greater than chance in all of the blocks after Block 3 (smallest significant t value Block 4: $M = 82.65$, $t\,(3) = 10.42$, $p = .002$). Finally for the TO-VR2 10-min group performance was only statistically greater than chance in Blocks 6 and 8 (smallest significant t value Block 6: $M = 86.86$; $t\,(3) = 7.23$, $p = .005$).

The addition of the VR2 10-min and TO-VR2 10-min groups to those from Experiment 1 did change some of the ANOVA results that were found in the first experiment. While there were still linear, quadratic effects, and Group effects, in this case there was also a linear Group X Block interaction and there was no Time of Day effect. Based on the slope of the learning curves it appears that the VR2 10-min group learned the task quickly, while the TO-VR2 10-min group did not learn the task. This was confirmed by the fact that the TO-VR2 10-min group was not consistently different from chance levels.

Skipped Session Probes

Classification of the probe trials followed the same procedure that was used in Experiment 1. For the rats in Experiment 2, on average the first probe trial was administered during Block 7. One rat in the TO-VR2 10-min group did not receive any probe trials because it failed to reach criterion. In the VR2 10-min group, two rats used a circadian strategy and one rat used an ordinal strategy. The strategy used by the remaining rat in this group could not be determined because it tended to chose the correct

lever following skipped morning sessions and to chose incorrectly following skipped afternoon sessions. Of the three rats that received probes in the TO-VR2 10-min group, one rat used an Ordinal strategy and the remaining two rats had the same pattern of results that could not be interpreted as one of the known strategies.

General Discussion

Whether it is concluded that the rats mastered the daily TPL task depends on which measure was used. The data were analyzed using three different measures: first arm choice, first lever pressed and percentage of presses to the correct lever prior to the first reinforcer (pre-reinforcement presses). The first arm choice data from both Experiments 1 and 2 suggest that the rats did not acquire the task. Only one of the 30 rats from both experiments achieved criterion. While it appears that the rats in Experiment 1 did improve on the task, none of the rats were statistically better than chance at any point in the experiment. Furthermore, when all of the groups from both experiments were added to the analysis, there was no linear effect for the Block factor, suggesting that there was no overall improvement in performance with training. Surprisingly in Experiment 1, the VR2 rats performed better than the TO-VR2 rats. Also, in Experiment 2 performance did not differ between the new 10-min groups and their counterparts from Experiment 1. The overall impression from the first arm choice data is that none of the groups successfully learned the task.

However, the conclusions about the ability of the groups to learn the task is quite different if one considers which lever the rats pressed first in each session. Of the 30 rats in the two experiments, 25 reached criterion in total. For example, all eight of the VR30 group, seven of the eight rats in the VR2 group, five of seven rats in the TO-VR2 group,

all four of the rats in the VR2 10-min group and two of the four rats in the TO-VR2 10-min achieved criterion. Significant linear and quadratic trends indicate that the performance of the rats improved with continued training. However, in both experiments the data generally suggest that the groups with a definite time-out (i.e., TO-VR2 and TO-VR2 10-min) performed worse than the other groups. For example, in Experiment 1, although the linear slope for the TO-VR2 group was greater than the VR2 group, this does not suggest that these rats performed better than the VR2 rats because in the first few blocks performance was much worse for the TO-VR2 group. For example, performance was not consistently above chance for the TO-VR2 group, while the performance of the VR2 rats exceeded chance levels after the third block. The VR2 rats also achieved criterion much faster than the TO-VR2 rats. With the addition of the 10-min trial lengths for the two groups in Experiment 2, the VR2 10-min group continued to show mastery of the task while the TO-VR2 10-min group gave no indication that they had learned the task.

Similar to the first press data the pre-reinforcement presses data also provide evidence to suggest that the rats acquired the task. Of the 30 rats in both experiments, 28 reached criterion and the only two rats that did not achieve criterion were in the TO-VR2 group. Based on the slopes of the linear trends the VR30 group appeared to have acquired the task the quickest. However, when examining the point at which the groups consistently performed better than chance and the mean number of trials to criterion the VR2 and VR2 10-min groups outperformed the VR30 group. Again, the groups with the time-out seemed to be impaired, particularly in the 10-minute condition.

Also, for several of the measures in both of the experiments there was a Time of Day effect, which indicated that the animals performed better in the afternoon than in the morning. This was the case for the first arm choice data in both experiments, and the first press and pre-reinforcement presses data from Experiment 1. There are several possible explanations for this outcome. First, the rats might be hungrier in the afternoon and thus are more motivated to receive reinforcement. Second, although the skipped session probe trials indicated that the rats were not using a nontiming alternation strategy, perhaps they were using the information from the morning session in conjunction with either the circadian or ordinal strategies. Thus, the animals might have learned that to receive reinforcement in the afternoon they had to press the opposite lever that provided reinforcers in the morning. Nevertheless, this effect was not evident for the first press or pre-reinforcment presses data when all of the groups were included in the analyses.

Perhaps one of the most intriguing outcomes from this study is the fact that the conclusion of whether the rats learned the task depends on the dependent measure used. If one considers the first arm that the rat chose it would be concluded that the rat had not learned the task. However, if either the first lever pressed or the proportion of presses on the correct lever prior to reinforcement was chosen as the dependent measure, it would be concluded that the majority of the rats solved the task. Using a similar paradigm, Mitslberger et al. (1996) also concluded that rats only learned the task if the pre-reinforcement presses data were considered, but not if the first arm chosen was considered.

Based on the results of the current study, it would appear that response cost of the task is not a major predictor of whether rats will learn the TPE discrimination. The

Response-Cost hypothesis would have predicted superior performance in the VR30 group compared to the VR2 group. However, the data suggest that this was not the case. As previously mentioned for the VR2 and VR2 10-min groups performance was statistically greater than chance sooner than the VR30 group, for both the first press and pre-reinforcement presses data. Also, when considering the first press data the rats in these groups achieved criterion sooner than the rats in the VR30 group. These results challenge the response cost theory for daily TPL. Response cost may have resulted in more "learning' in some studies (Lukoyanov et al., 2002; Widman et al., 2000; Widman et al., 2004), not because the rats were actually learning the time-place-event code better, but because the higher response cost or task difficulty inhibited species typical behaviour.

Another possibility is that the Response-Cost theory may only apply once a minimum amount of difficulty is surpassed. Thus, the idea that response cost exists on a continuum and that there is a positive correlation between response cost and learning, might not be the case. Rather, once the response-cost exceeds a cut-off point, learning will occur. In the present study, possibly the VR2 schedule provided a sufficient amount of task difficulty for learning to occur. While it is possible that the VR2 exceeds the lowest level of difficulty necessary, it seems unlikely that pressing on a VR2 schedule is that much more difficult than walking down the arm of a T-maze.

However, if the failure of rats to learn daily TPL tasks is due to the intrusion of species typical behaviours, then the groups with a definite time-out (TO-VR2 and TO-VR2 10-min) should have performed better than the other groups. Carr and Wilkie (1997b, 1999) successfully implemented time-out periods in their operant box studies of daily TPL. However, in the present study the time-outs did not increase the performance

of the rats. In the paradigm used in the current study, the 2-min time-out period was possibly too long and the rat's pressing on the correct lever during the time-out was extinguished. For example, Carr and Wilkie (1997b, 1999) found that a 10-s time-out period after the first lever press (ranged from 14-50-s from the start of the session), successfully controlled for species typical behaviour. In future research it would be interesting to compare a group with a shorter time-out period to the groups from the present study with 2-min time-outs.

While the time-out groups did not out-perform the other groups as predicted, evidence for the Species-Typical Behaviours hypothesis comes indirectly from the discrepancies between the first arm choice and first press data. The rat's failure to choose the correct arm was not due to a failure in learning, but rather due to the tendency of the rats to patrol the maze. If one ignores the first arm that the rats chose and instead looks at the lever first pressed, it is evident that the rats learned the task. Therefore the first press and pre-reinforcement presses are a more accurate measure of what the animal has learned because the first arm choice is confounded by species typical patrolling behaviour.

If the first arm that rats chose in a daily TPL task is not a good indicator of whether they learned the task, then it is not surprising that many of the previous studies examining daily TPL have also failed. For example, Means, Ginn, Arolfo, and Pence (2000) demonstrated that although rats could learn a daily TPL task in the T-maze (no levers), it took many trials, with only 63 % of the rats' acquiring the task. Similarly, in the studies by Thorpe et al. (2003) and the low response-cost radial arm maze task of White and Timberlake (1990), the dependent measure was the first location chosen by the

rat. It is possible that if a different dependent measure was chosen, that is, one in which the rats could patrol the apparatus first, it might have been concluded that the rats learned the task.

Although the Species-Typical Behaviour hypothesis explains the present set of results and many of the previous unsuccessful daily TPL results, this hypothesis has difficulty explaining two important pieces of data. First, the Species-Typical Behaviour hypothesis does not seem to apply to similar fields, such as spatial learning. For example, in successful spatial learning studies (e.g., Skinner, Etchegary, Ekert-Maret, Baker, Harley, Evans, & Martin, 2003) the animal's first arm choice data show evidence of learning. It is unclear why species typical behaviour might interfere in daily TPL, but not in similar spatial learning tasks. Second, as described previously Thorpe and Wilkie (2007) demonstrated that rats were able to learn a low response cost task in the T-maze that provided different amounts of food depending on the time of day, even when only the first arm choice data were considered.

The bipartite code explanation used to explain the Thorpe and Wilkie (2007) findings may also be used to explain the results of the current study (see Figure 1). They hypothesized that rats do not typically use a single tripartite (time-place-event) memory code, but instead use two bipartite memory codes (time-event and event-place) and this is why animals fail to learn many daily TPL tasks. When the amounts of food (event) are the same, each place has an equal association with the event and the bipartite codes are not able to mediate successful performance (Refer to Figure 1A). However, when the events were different (1 Fruit Loop (FL) vs. 1/5 FL) the bipartite codes were more distinguishable and thus the animals acquired the task (Refer to Figure 1B).

In the current study, when the rats are placed on the start arm they cannot use time of day as a discriminative stimulus to tell them where to go. Instead, it uses time to tell it whether food will be available (e.g., T_1-E_{food} and T_2-E_{food}). Based on previous exposures to the maze it also knows which places are associated with food (e.g., E_{food}-P_1 and E_{food}-P_2). If, for example, the rat is placed on the start arm in the morning it can recall that T_1 is associated with food, but food has previously been found in both P_1 and P_2. Therefore it will randomly choose between the two arms. This is in fact what the rats in the current study did.

Based on the results of the current study it is further proposed that the reason the rats can successfully chose the correct lever is that when it is at the end of the choice arm (i.e., in front of the lever) it has all three components of the tripartite code available to it. For example, it knows that it is T_1 and that it is in P_1. It can then examine its codes to see if that particular combination has been reinforced in the past (i.e., T_1-E_{food}-P_1 versus T_1-$E_{no\ food}$-P_1). If it has been associated with food, the rat will press the lever; if not, it will go to the next location that has been associated with food. This is in line with the Occasion setting explanation proposed by Means, Ginn, Arolfo, and Pence (2000) which suggested that the poor results displayed in many of the daily TPL tasks occur because rats might not be able to use time as a discriminative stimulus when in the start location, but instead time might act as an occasion setter when in the correct location.

Thorpe and Wilkie (2007) speculated that making the task more difficult by increasing the response cost enables the formation of tripartite memory codes. However, the current proposal that the rats have access only to bipartite codes at the start arms and can make use of tripartite codes only when they are in the location at the time can also be

used to explain the results of the high response cost tower maze study (Widman et al., 2000). When the rats are placed on the maze they do not know which of the locations contains food at that particular time. By patrolling the maze, they are able to go to the locations that provide food. Once they are in the correct location for that time of day they have access to the tripartite code and they can make the response of climbing the appropriate tower. Because the dependent measure is the tower climbed, it is concluded that the rats have learned the task. It is possible that if they had measured the direction that the rats went in when placed on the maze, that they would not have found that the rats went directly to the correct place at the correct time of day.

This logic might not apply to the studies in the water maze (Lukoyanov et al., 2002; Widman et al., 2004). In those studies the animals only acquired the task if the response cost was increased by severely food depriving the rats (Lukoyanov et al., 2002) or by adding weighted vests to the animals (Widman et al., 2004). It is possible that the bipartite theory still applies to the standard water maze task because normal rats cannot acquire this task (Lukoyanov et al., 2002; Thorpe et al., 2003; Widman et al., 2004). But, when these tasks were made more difficult, it appears that tripartite memory codes mediated successful performance.

Several follow-up studies present themselves based on the proposed hypothesis. For example, a replication of the Thorpe and Wilkie (2007) differential food study could be conducted in the present paradigm such that in one of the daily sessions the amount of food delivered would be greater than the amount of food delivered in the other session. For example, each reward would consist of five pellets in the lots of food session, compared to one pellet in the little amount of food session. If the rats can make use of the

different event codes in the bipartite codes to solve the task then the rats' first arm choice data should indicate task acquisition, in addition to the first lever press and pre-reinforcement presses data. If this were the case, these findings would indicate that controlling for species typical behaviours is not important. Instead these results would support the idea that indistinguishable bipartite memory codes are used in the start arm and this is why the rats first arm choice performance is poor. However, if the proposed study does not find improved first arm choice performance, then the Species-Typical Behaviour theory for daily TPL still needs to be investigated.

It would also be useful to conduct a study to determine if time of day can be used as a discriminative stimulus in non-spatial tasks. One way to test this would be to have rats respond to one lever such that there was one fixed interval schedule in morning sessions and a different fixed interval schedule in afternoon sessions. Probes tests could then be used to determine if rats can in fact use time of day as a discriminative stimulus.

To better understand how memories are encoded it is important to determine whether rats easily acquire daily TPL tasks and species typical behaviours overshadow performance, or instead, rats do not readily make time-event-place associations. If it is the case that species typical behaviours prevent researchers from seeing evidence for successful learning, then with procedural modifications, animals might be able to acquire daily TPL tasks in previously unsuccessful paradigms, such as the T, radial, and water mazes.

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(A) Normal Daily TPL study (T-E and E-P Bipartite codes)

Memory		*Decision*	
T_1-E_{food}	E_{food}-P_1	T_1-E_{food} →	E_{food}-P_1
T_2-E_{food}	E_{food}-P_2		E_{food}-P_2

Both P_1 and P_2 are equally associated with the E_{food}, so the animals randomly distribute their choice among the two places.

(B) Differential Food Daily TPL Study (Thorpe & Wilkie, 2007) (T-E and E-P Bipartite codes)

Memory		*Decision*	
T_1-$E_{1/5FL}$	$E_{1/5FL}$-P_1	T_1-$E_{1/5FL}$ →	$E_{1/5FL}$-P_1
T_2-E_{1FL}	E_{1FL}-P_2		

Each place is only associated with the corresponding event, so the animals got to the appropriate place for that time.

(C) Present study

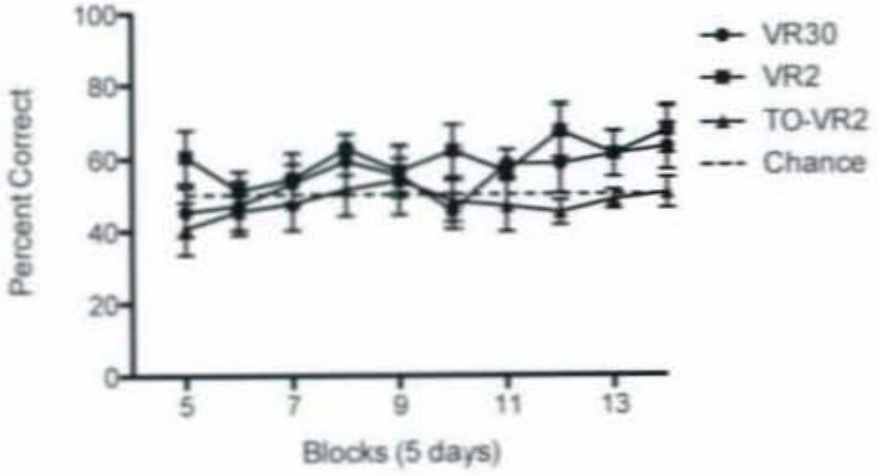
When in one of the arms of the T-maze (T-E-P Tripartite codes)

Memory	*Decision*
T_1-E-P_1	T_1-E-P_1
T_2-E-P_2	

When the animal is in the time and place simultaneously it is able to use tripartite memory codes. But, when in the start arm it is only able to use bipartite memory codes as in diagram A.

Figure 1. The bipartite and tripartite theories for various daily TPL studies. T, time; E, event; P, place; TPL, time-place learning. Figure adapted from the Thorpe and Wilkie (2007) study.

(A)



(B)

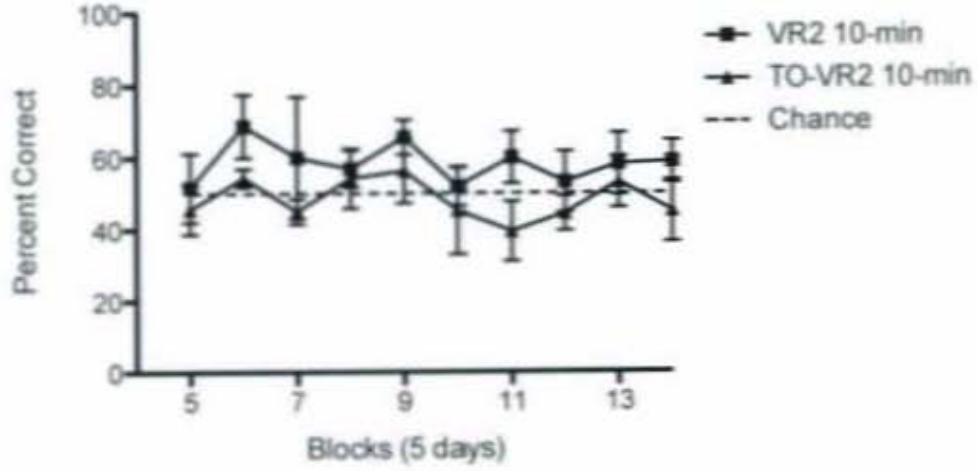
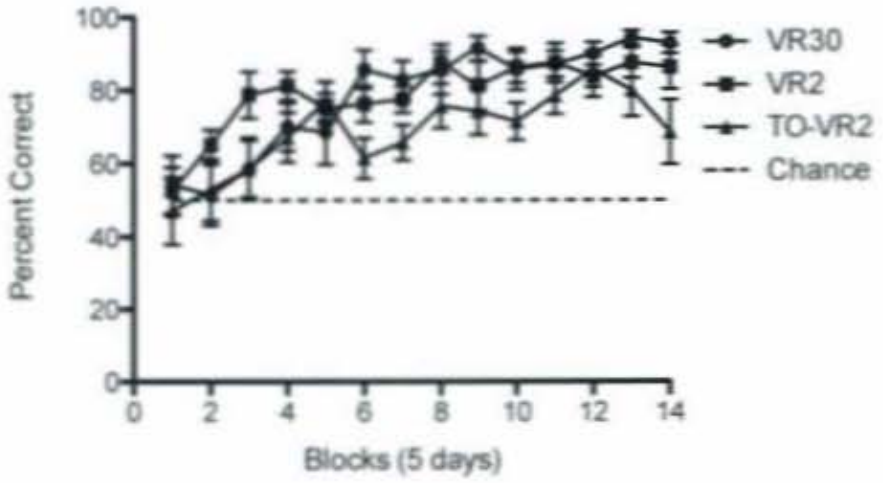


Figure 2. The rats' percentage of correct first arm choices from both Experiment 1 (A) and Experiment 2 (B) for the final 10 blocks of the experiments (chance was 50 %). The errors bars represent the standard error of the mean.

(A)



(B)

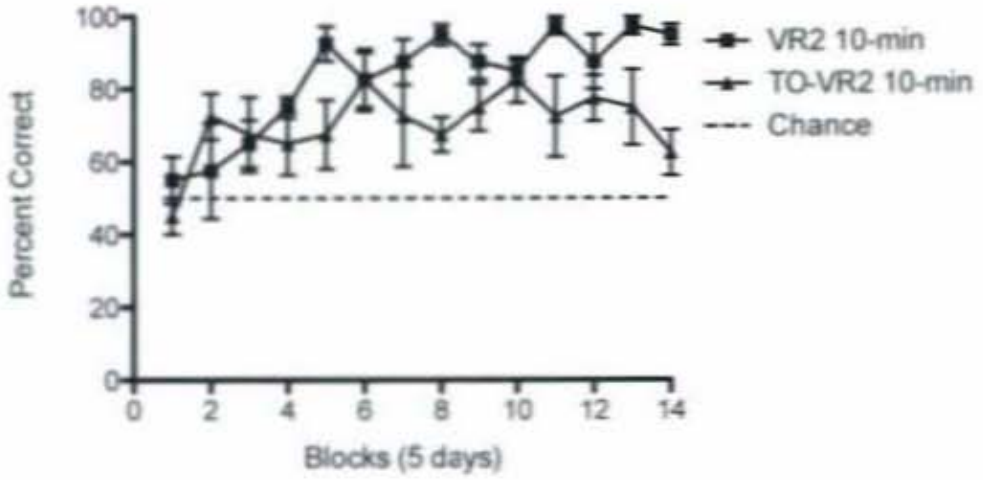
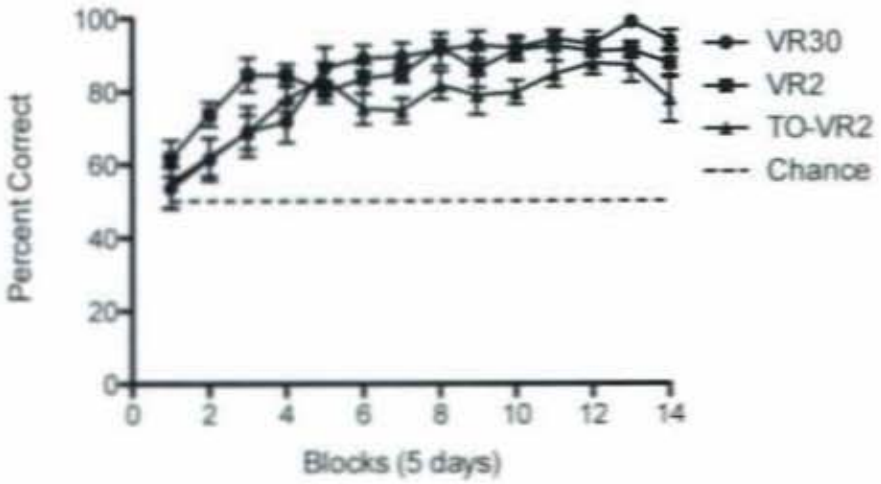


Figure 3. The rats' percentage of correct first presses for the entire 14 blocks of the study from both Experiment 1 (A) and Experiment 2 (B) (chance was 50 %). The errors bars represent the standard error of the mean.

(A)



(B)

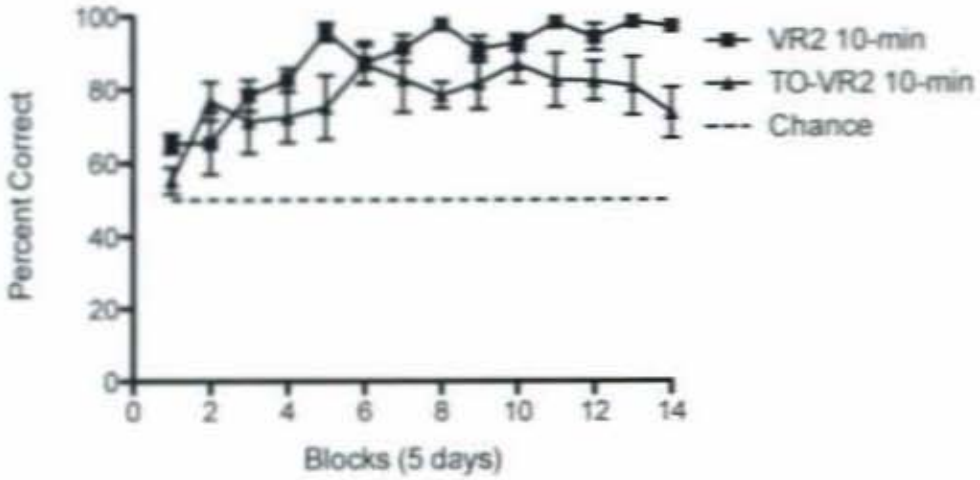


Figure 4. The rats average percentage of presses on the correct lever before reinforcement for the entire 14 blocks of the study from both Experiment 1 (A) and Experiment 2 (B) (chance was 50 %). The errors bars represent the standard error of the mean.